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Crystal Structure of Intramembrane Protease GlpG

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Intramembrane proteolysis was initially a controversial concept because proteolytic reactions, which involve water, usually take place in aqueous solutions instead of within cell membranes. Now we know that intramembrane proteolysis is common in biology, and is responsible for generating amyloid β-peptide that causes Alzheimer's disease. The crystal structure of GlpG describes for the first time with atomic resolution the architecture of one membrane protein that specializes in catalyzing this reaction, and illustrates the physical principles that underlie its unique mechanism.

Escherichia coli GlpG is an integral membrane protein that belongs to the rhomboid protease family. The crystal structure of GlpG core catalytic domain has been solved at 2.1 Å resolution based on x-ray diffraction data collected at the NSLS.

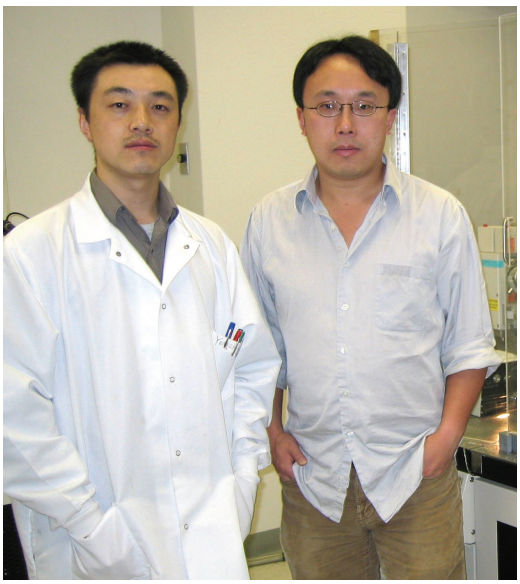
The structure of GlpG contains six transmembrane helices (**Figure 1**). Inside the bundle of transmembrane helices is a cavity (above the yellow helix S4). The cavity opens toward the outside of the cell, and is tightly capped by the L5 loop. The L1 loop appears to be embedded in the outer leaflet of the membrane as judged by its position relative to the transmembrane helices.

The crystal structure correlates well with published sequence and functional data on related rhomboid proteases (**Figure 2**). The internal cavity harbors all the polar residues from the transmembrane segments (His-150, Asn-154, Ser-201, His-254). A fully extended L3 loop, as well as the exposed N-terminus of helix S4, also contributes polar groups to the cavity. These features suggest that the cavity represents the active site of the protease. Substituting each of the conserved Gly-199, Ser-201, and His-254 invariably abolishes activity (colored red in the figure). Mutations of His-150 and Asn-154 also affect the activity for some rhomboid proteases, but not all (orange).

The active site is surrounded by five transmembrane helices. Between helices S1 and S3 sits the interesting L1 loop. The function of the loop in enzyme mechanism is not yet clear. Mutating Trp-136 or Arg-137, two preferred residues on L1 and 15 Å away from the active site, either abolishes or reduces protease activity (yellow in the figure).

The electron density map reveals several water molecules present in the cavity (**Figure 3**). When the L5 loop "opens," water can diffuse easily into the active site and react with substrate. These observations solve the long-time puzzle of a water-requiring reaction in the middle of cell membranes.

The crystal structure of GlpG illustrates the following physical principles underlying the mechanism of intramembrane proteolysis: the active site of the protease is positioned into the membrane at a distance roughly matching the cleavage site of the substrate; the hydrophilic active site is separated from the lipid by protein structures; substrate binding triggers conformational changes in the protease, which causes the L5 cap to open; substrate enters the protease laterally from its own transmembrane location and becomes hydrolyzed.



Yongcheng Wang (left) and Ya Ha

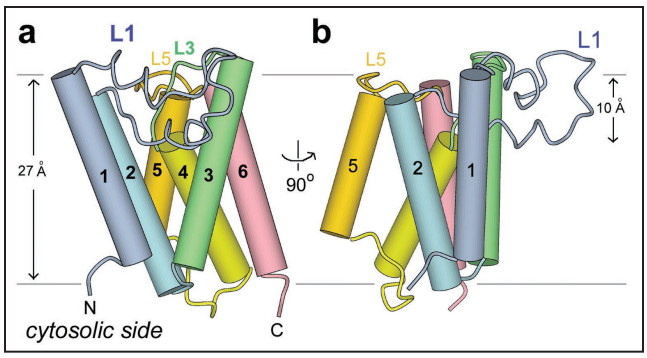


Figure 1. *The overall structure. (a) The front view. The transmembrane helices shown as cylinders are sequentially labeled 1-6. The two horizontal lines mark the boundaries of the membrane. (b) The side view related to (a) by a 90-degree rotation as shown.*

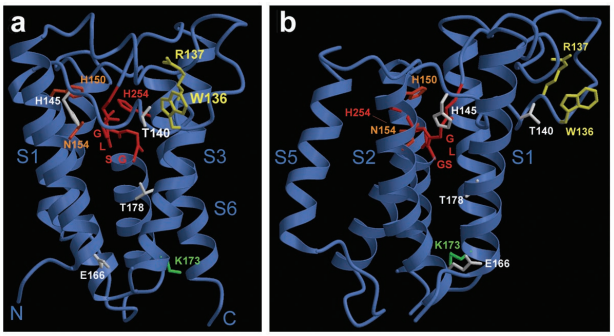


Figure 2. *Mutagenesis studies on related rhomboid proteases mapped onto GlpG structure. The GLSG (Gly-199, Ser-201) sequence motif and His-254 are shown in red; His-150 and Asn-154 in orange; Trp-136 and Arg-137 in yellow. (a) and (b) represent different views.*

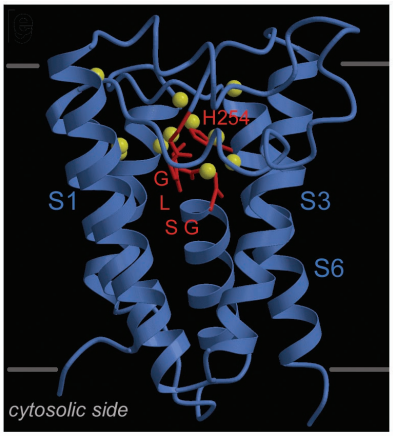


Figure 3. *The active site is filled with water (yellow spheres).*